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(56) Documents cited

The Extra Pharmacopoeia,
Martindale (27th Edition),
pages 212—213, 651—

652, 738—745, 1275—6,

1279, 1828. The Merck

Index (9th Edition),

Monographs 43, 1100,

2307, 3021, 5185, 5186,

7615, 8093, 8845

(58) Field of search

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(54) Use of carboxylic acids as
virucides

(57) Methods, compositions and
products for interrupting or preventing
the spread of harmful respiratory
viruses and so inhibiting the spread of
diseases, including the common cold,
in which one or more carboxylic acids
such as citric, malic, succinic and
benzoic acids are applied in an
effective amount optionally together
with a surfactant, to a virus-containing

area, such as human tissue surfaces.

Application may be by means of
impregnated or coated substrates
such as facial tissue, nonwoven
materials, and the like. In one
application, treated tissue, when
substituted for ordinary facial tissue
and used in wiping the nasal area of a
person suffering from a virus-borne
infection is effective in annihilating the
virus on contact with the treated
tissue, and, in turn, preventing the
spread of the virus-related illness.

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SPECIFICATION

Virucidal method, composition and product

- This invention relates to a class of virucidal compositions highly efficacious against common respiratory viruses such as rhinoviruses, parainfluenza viruses, and adenoviruses and the methods and products utilizing such compositions. In particular, the invention relates to a novel type of virucidal composition which can be applied to a variety of substrates such as cellulosic webs, nonwoven structures, and textile-based materials. In addition, the virucidal compositions of this invention may also be incorporated into nasal sprays, facial creams, hand lotions, lipsticks, and similar cosmetic preparations. The compositions may also be used as ingredients in kitchen and bathroom cleansers, furniture and floor polishes, and similar household preparations.
- Virologists knowledgeable in the field of respiratory viruses generally agree that rhinoviruses, influenza viruses, and adenoviruses are among the most important group of pathogenic agents which cause respiratory illnesses. Rhinoviruses, in particular, are thought to be the principle causative agent of what is generally known as "the common cold".
- The term "rhinovirus" reflects the involvement of the nose resulting in copious nasal discharges when infections are caused by this group of viruses. Rhinoviruses belong to the picornavirus family of viruses which, lacking an outer envelope, are often characterized as "naked viruses". Although more than 100 different antigenic types of rhinoviruses are known, they share certain centrally important attributes. For instance, all are endowed with ether-resistant capsids and all contain single-stranded RNA (ca. 2.6×10^6 daltons). All are difficult to inactivate by common germicides such as quaternary ammonium compounds.
- Adenoviruses include more than thirty antigenic types. When they invade the respiratory tract, they cause inflammation of the tissues leading to symptoms of pharyngitis, bronchitis, etc. While most adenovirus infections occur in childhood, infections of adults are far from uncommon. Like rhinoviruses, adenoviruses lack an envelope, but the adeno-nucleus, in contrast to the rhino-nucleus, contains a double-stranded DNA. Adenoviruses are unusually resistant to inactivation.
- Parainfluenza viruses belong to the paramyxovirus family. They play an important role in the occurrence of lower respiratory diseases in children and upper respiratory diseases in adults. The parainfluenza viruses are RNA-containing viruses endowed with an ether-sensitive lipoprotein envelope surrounding the nucleocapsid. These viruses are resistant to inactivation by carboxylic acids in low concentrations.
- Recent work by Dick and others [Dick, E. C. and Chesney, P. J., "Textbook of Pediatric Diseases", Feigin, R. D. and Cherry, J. D. ed., Vol. II, p. 1167 (1981) W. B. Saunders Pub. Co., Phila., PA] has thrown considerable light on the mode of transmission of respiratory diseases caused by rhinoviruses. Although the exact mode of transmission of respiratory diseases is not fully understood, field studies by the above investigators have provided persuasive evidence that effective transmission of diseases such as common colds usually requires close association or contact—direct or indirect—between the infected subject and the potential victim. (Indirect contact may be looked upon as contact occurring via an intervening surface, e.g., table top, door knob, etc.). Thus, it may be possible to interrupt the chain of infection and reduce its potential to spread, if the viruses can be rendered ineffective as they emerge from an infected person's nose or mouth by immediate exposure to a virucidal agent. Moreover, after emergence, viruses which may ensconce themselves on the infected person's face or hands may also be "killed" if a suitable virucidal agent is quickly brought into contact with the appropriate anatomical surface, i.e., face, hands, etc. A facial tissue, containing a fast-acting, efficacious virucidal composition would offer a simple means of accomplishing the tasks mentioned above.
- A long-felt need has existed for a safe and inexpensive virucidal agent effective against common respiratory viruses. Simple household germicides are not effective against rhino- and adenoviruses. U.S. Patent 4,045,364 to Richter discloses a disposable paper impregnated with an iodophor (i.e. iodine and a carrier) having germicidal properties and useful as a pre-wash in a surgical scrub routine. The patentee discloses that the stability of the iodophor is enhanced at a lower pH and that small quantities of weak organic acids such as citric acid or acetic acid can be added to achieve pH control. U.S. Patent 3,881,210 to Drach *et al* describes a pre-moistened wiper for sanitary purposes which can include a bactericide. U.S. Patent 3,654,165 to Bryant *et al* discloses a cleaner/sanitizer for wiping purposes including iodine providing bactericidal action. U.S. Patent 3,567,118 to Shepherd *et al* discloses a fibrous material for cleaning purposes having a coating of a hydrophilic acrylate or methacrylate containing, *inter alia*, a bactericide.
- While the prior art has disclosed that iodine compositions and products have a wide-spectrum virucidal effect, there has yet to be developed commercially an inexpensive product that successfully interrupts the spread of viruses such as rhinovirus or influenza virus. Problems with iodine result, for example, from its toxicity, and the fact that it is an irritant for animal tissue. The action of iodine is non-selective as between bacterial and mammalian protein, and its uncontrolled use upon the skin may cause severe irritation. Further, its activity may be diminished or neutralized by the action of biological fluids such as blood serum. Efforts to modify iodine to avoid these difficulties have not been completely successful.

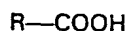
References exist in the literature on the bactericidal action of acids such as citric, [e.g., Reid, James D., "The Disinfectant Action of Certain Organic Acids", *American Journal of Hygiene*, 16, 540—556 (1932)]. However, virucidal action is fundamentally different from bactericidal action in that viruses and bacteria represent different microorganisms with different characteristics. For instance, viruses do not replicate outside host cells whereas bacteria do. Quaternary ammonium compounds such as benzalkonium chloride are often effective against bacterial but not against viruses such as the various rhinoviruses.

Although it is known that rhinoviruses are labile to aqueous solutions of acids under low-pH conditions [e.g. Davis, B. D. et al: "Microbiology" p. 1303. Harper E. Row (Publishers) New York, 1973 and Rueckert, R. R., "Picomaviral Architecture" Comparative Virology—Academic Press, New York (1971), pp. 194—306], known references do not mention the utilization of this concept in epidemiological contexts such as interruption of the chain of infection caused by rhinoviruses. To the best of our present knowledge the only systematic study of the virucidal action of organic acids (citric, malic, etc.) which exists in the generally available literature, was carried out by Poli, Biondi, Uberti, Ponti, Balsari, and Cantoni [Poli, G. et al: "Virucidal Activity of Organic Acid" *Food Chem.* (England) 4(4)251—8 (1979)]. These workers found that citric, malic, pyruvic and succinic acids, among others, were effective against herpesvirus, orthomyxovirus and rhabdovirus (Rabies virus). Their experiments were carried out at room temperature with aqueous solutions of pure acids. No substrate or carrier was used. The three viruses chosen for study by these workers were all "enveloped" viruses, resembling, in that regard, parainfluenza 3. Poli et al also observed that these acids were not effective against adenovirus which, it will be recalled, is a "naked" virus. Based on this, they concluded that these acids were effective against "enveloped" viruses but not against "naked" viruses.

It is known to those skilled in the art that adenoviruses are resistant to acids.

We have now surprisingly found that certain carboxylic acids, such as citric, malic, and succinic, deployed in an appropriate physiologically acceptable carrier, in addition to being effective against certain respiratory "enveloped" viruses, are also effective against rhinovirus, a "naked virus" and, in the presence of a surfactant such as sodium dodecyl sulfate, are also effective against adenovirus.

According to one aspect of the invention there is provided a method for interrupting or preventing the spread of respiratory viruses which comprises contacting a virus-containing area with a virucidally effective amount of a virucidally active composition comprising one or more virucidal acids which are essentially non-toxic and non-irritating to human or animal tissue selected from acids of formula



(wherein R represents a lower alkyl, substituted lower alkyl, carboxy lower alkyl, carboxy hydroxy lower alkyl, carboxy halo lower alkyl, carboxy dihydroxy lower alkyl, dicarboxy hydroxy lower alkyl, lower alkenyl, carboxy lower alkenyl, dicarboxy lower alkenyl, phenyl or substituted phenyl group).

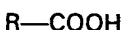
According to a further aspect of the invention there is provided a virucidal composition comprising a physiologically acceptable carrier containing a virucidally effective amount of one or more acids which are non-toxic and non-irritating to human or animal tissue in the amount used selected from acids of formula



(wherein R represents a lower alkyl, substituted lower alkyl, carboxy lower alkyl, carboxy hydroxy lower alkyl, carboxy halo lower alkyl, carboxy dihydroxy lower alkyl, dicarboxy hydroxy lower alkyl, lower alkenyl, carboxy lower alkenyl, dicarboxy lower alkenyl, phenyl or substituted phenyl group).

In addition the compositions of the present invention may be incorporated into a substrate such as facial tissue or a nonwoven web.

According to a still further aspect of the invention there is provided a virucidal product comprising a web substrate containing a virucidally effective amount of a composition comprising one or more acids which are non-toxic and non-irritating to human or animal tissue in the amount used and are selected from acids of formula



(wherein R represents a lower alkyl, substituted lower alkyl, carboxy lower alkyl, carboxy hydroxy lower alkyl, carboxy halo lower alkyl, carboxy dihydroxy lower alkyl, dicarboxy hydroxy lower alkyl, lower alkenyl, carboxy lower alkenyl, dicarboxy lower alkenyl, phenyl or substituted phenyl groups).

In a preferred embodiment of the method, virucidal composition and product of the invention, the composition comprising one or more acids further comprises a surfactant selected from nonionic, cationic and anionic surfactants.

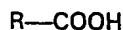
In the method of the invention an effective amount of such a composition is brought into contact with the affected area, preferably through the use of the virucidal products according to the invention. In general, the virucidal compositions can be handled without difficulty and are not believed to have

any harmful effects when used in accordance with the invention. In addition, when applied to a substrate such as facial tissue, the compositions have little or no deleterious effects on color, odor, strength, or other important properties. The virucidal product according to the invention may be used as a dry wipe or maintained moist and used as a wet wipe, for example.

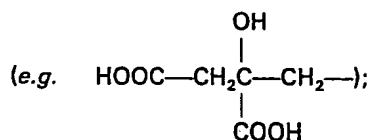
- 5 Thus the present invention provides a virucidal product, composition and method which are highly effective over a broad spectrum of viruses and yet can be produced and used with safety. 5

While the invention will hereinafter be described in connection with preferred embodiments, it will be understood that it is not intended to limit the invention to those embodiments.

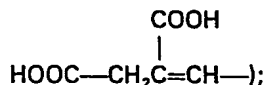
- 10 The present invention results from the unexpected discovery that certain acids such as citric, malic, succinic, and benzoic, used in suitable concentrations are as further described herein, highly efficacious against rhinoviruses 16, 1A, and 86. When used in the presence of a surfactant such as sodium dodecyl sulfate (SDS) these acids were found to be effective also against parainfluenza 3 and adenovirus 5. (The particular viruses chosen for study, i.e., RV-16, RV-1A, RV-86, Para-3, and Adeno-5, are representatives of their classes). In general, the water soluble carboxylic acids useful in 15 accordance with the invention have the following structure: 15



- Wherein R may be represented by: lower alkyl (one to six carbon atoms); substituted lower alkyl [e.g. hydroxy lower alkyl (e.g. $HOCH_2-$); carboxy lower alkyl (e.g. $HOOC-CH_2-CH_2-$); carboxy, hydroxy lower alkyl (e.g., $HOOCCH_2CHOH-$); carboxy halo lower alkyl (e.g. $HOOCCH_2CHBr-$); carboxy 20 dihydroxy lower alkyl (e.g. $HOOC-CHOH-CHOH-$); dicarboxy, hydroxy lower alkyl 20



lower alkenyl, carboxy lower alkenyl (e.g. $HOOCCH=CH-$); dicarboxy lower alkenyl (e.g.

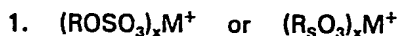


- 25 phenyl (i.e. C_6H_5-); or substituted phenyl (e.g. hydroxy phenyl $HO-C_6H_4-$). Other examples of R and the acids $R-COOH$ include hydroxy lower alkyl, lactic, carboxy, hydroxy lower alkyl, 2-methyl malic; carboxy, halo, lower alkyl, 2-chloro-3-methyl succinic; carboxy dihydroxy lower alkyl, 2-methyl tartaric; dicarboxy, hydroxy lower alkyl, 2-methyl citric acid; and carboxy lower alkenyl, fumaric. The above definitions are used in an illustrative but not a limiting sense. The term "substituted" indicates that one or more hydrogen atoms are substituted by halogen atoms (F, Cl, Br, I) or groups such as hydroxyl 30 groups, amino groups, thiol groups, nitro groups, and cyano groups. 30

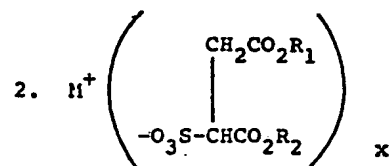
The surfactant may be nonionic (e.g., the polyoxyethylenated alkylphenols such as Triton X-100®, manufactured by Rohm and Haas; the polyoxyethylenated sorbitol esters such as Tween 40®, manufactured by ICI United States, Inc.), cationic (e.g. cetylpyridinium chloride ($C_5H_5N^+(CH_2)_{15}CH_3 Cl^-$), methylbenzethonium chloride

- 35 $(Me_3CCH_2C(Me)_2-C_6H_3(Me)-OCH_2CH_2OCH_2-CH_2N^+(Me)_2-CH_2C_6H_5 Cl^-)$ 35

or anionic (e.g., sodium dodecyl sulfate ($CH_3(CH_2)_{10}-CH_2OSO_3^-Na$), the 1,4-bis (2-ethylhexyl) ester, sodium salt of sulfosuccinic acid, as manufactured by American Cyanamid Company under the tradename of Aerosol OT. The preferred anionic surfactants may be represented by the structures:



- 40 Wherein, M^+ is a mono, di or trivalent metal cation or an ammonium or substituted ammonium ion; x is an integer; and R is an alkyl group. 40



Wherein, M^+ and x are defined as above and R_1 and R_2 may be the same or different and may be represented by straight or branched chain aliphatic groups. The above anionic surfactants are presented in an illustrative rather than a limiting sense. Surfactants, in general, are not, alone, virucidal with respect to naked viruses such as rhinovirus.

- 5 In general in the acids and surfactants of the compositions of the invention, the groups, R , R_1 or R_2 where they represent lower alkyl or alkenyl groups represent groups having a carbon atom content of about 1 to 6. 5

Although the invention is not limited to the use of a cellulosic web (such as facial tissue, bathroom tissue, hand towels for washroom and other uses and the like) as the substrate or carrier for the virucidal agents, a facial tissue impregnated with these novel virucidal compositions sufficiently illustrates the underlying principle and represents a simple and useful embodiment of the invention. For this reason, the experiments described in the paragraphs which follow were carried out using facial tissues as the substrate. Examples of suitable nonwoven substrates are wet wipe materials such as wet-creped hand towels and spunbonded and meltblown polymeric webs commonly used in production of disposable hospital items such as surgical drapes, gowns, bedsheets, pillowcases, and the like. Textile materials of all types, including laminates of different materials may be used as suitable substrates. For example, hygienic face masks used by persons suffering from respiratory illnesses provide an excellent means for utilizing the present invention. Other physiologically acceptable, essentially inert carriers i.e., those which are essentially non-toxic and non-irritating to human or animal tissue under the conditions of normal use, will be apparent to those skilled in the art for applications such as lotions, sprays, creams, polishes and the like. 10 15 20

The virucidal efficiency of the method, composition and product of the invention are illustrated by the following Examples.

In general terms, the experimental procedure for preparing the samples in the examples below was simple and straightforward. Three-ply Kleenex facial tissues (11 inches x 12 inches (27.9 x 30.5 cm); basis weight: ca. 26 lb/2880 ft.² (0.044 kg m⁻²) for all three plies combined) were impregnated with aqueous solutions of citric, malic, succinic, and benzoic acids by simple dipping. The acids were used either singly or as homogeneous mixtures. Usually the impregnating solution also contained a small percentage of a surfactant such as Aerosol-OT—[sodium salt of 1,4-bis(2-ethylhexyl) ester of sulfosuccinic acid, manufactured by American Cyanamid], or sodium dodecyl sulfate. In certain instances, a small amount of glycerol was also used to enhance tissue softness. The saturated tissues were pressed between rolls to squeeze out excess saturant and ensure uniformity of saturation. The tissues were weighed, dried, and the degree of saturation (i.e. percent saturant pick-up) was computed. The tissues were then ready for the testing of virucidal efficacy. 25 30

The procedure adopted for testing virucidal efficacy is in accord with standard virological assay techniques (TCID₅₀) with simple variations necessitated by the presence of the cellulosic substrate. A description of the procedure follows: 35

Virucidal assay procedure

I. Materials:

40 A. Solutions: 40

1. Neutralizing solution

6.4 ml 2M Na₂HPO₄

1.2 ml 1.0 M Citric Acid

92.4 ml 1X Medium 199 (nutrient medium for tissue culture)

45 2. Hanks'—McIlvaine salt solution (HMSS): 45

2.0 ml 1.0 Citric acid

18.0 ml 2.0 M Sterile Na₂HPO₄

The pH of this solution is 7.0

Diluted to 2 liters with Hanks' Balanced salt solution

3. Hanks' balanced salt solution:

50 *g/liter in double-distilled water* 50

NaCl

8.0

KCl

0.4

MgSO₄·7H₂O

0.2

CaCl₂ (anhydrous)

0.14

55 Na₂HPO₄·2H₂O 0.06 55

KH₂PO₄ (anhydrous)

0.06

Glucose

1.0

Phenol red

0.005

NaHCO₃

0.35

60 Note: The above solutions are not virucidal. 60

B. Viruses and tissue culture cell lines:

1. Rhinovirus type 16, type 1A and type 86:

Rhinovirus types 16, 1A, and 86 (RV 16, 1A and 86 respectively) are grown in Ohio State HeLa (O-HeLa) tissue culture cells and stored at -60°F (-51.1°C) until they are used. The virucidal testing involving the rhinoviruses is done using O-HeLa tissue culture test tubes incubated on a roller drum apparatus at 33°C .

2. Parainfluenza type 3:

Parainfluenza type 3 (para 3) grown in rhesus monkey kidney tissue culture cells and stored at -60°F (-51.1°C) until it is used. The virucidal testing involving Para 3 virus is done using O-HeLa tissue culture test tubes incubated in a stationary position at 33°C .

3. Adenovirus type 5:

Adenovirus type 5 (Adeno 5) is grown in HEp-2 tissue culture cells and stored at -60°F (-51.1°C) until it is used. The virucidal testing involving adeno 5 virus is done using Human Epithelial Carcinoma—2 (HEp-2) tissue culture test tubes incubated in a stationary position at 37°C .

II. Methods

A. Virucidal testing

A 1:1 (volume:volume) mixture of virus and saliva is prepared. A one-square inch ($2.5\text{ cm} \times 2.5\text{ cm}$) sample is cut out of treated Kimberly-Clark Kleenex® tissue and placed in a plastic petri dish. (A treated tissue is tissue impregnated with the virucidal agent under investigation). The virus—saliva mixture (0.1 ml) is pipetted directly onto the sample and allowed to react for one minute. Note this is a two-fold virus dilution. After the reaction time of one minute, 5 ml of neutralizing solution is pipetted onto the sample in the petri plate and agitated for 3 seconds. This is now a 100-fold virus dilution. The neutralizing solution—virus—saliva mixture is then pipetted out of the petri plate and added to a tube containing 5 ml of Hanks'—McIlvaine Salt Solution. The sample is added to the same tube by tipping the plate and using the tip of a pipette to push it into the tube. The cube containing the 10 ml of solutions and the sample is vortexed for 30 seconds. This tube contains a $10^{-2.3}$ or 1:200 dilution of virus. Ten-fold serial dilutions (fresh pipette for each dilution) are made from the $10^{-2.3}$ dilution by taking 0.3 ml of the previous dilution and adding it to 2.7 ml of Hanks'—McIlvaine Salt Solution. 0.1 ml is inoculated into each tissue culture test tube. Generally two tubes are inoculated per dilution.

For each experiment two sets of controls are used. The first may be termed "the virus control" as it is designed to check the infectivity of the virus suspension itself without saliva or the tissue substrate. The virus suspension is diluted serially 10-fold in HMSS. 0.1 ml of specific dilutions are inoculated per tissue culture cell test tube. The information obtained from this control gives the number of infectious virus units that are contained in the virus solution that has been stored at -60°F (-51.1°C) and insures that the aliquot of virus solution used in the experiment has not lost infectivity during the freezing, storage, or thawing processes.

The second control, "the tissue control", consists of performing the virucidal testing experiment using one square inch ($2.5\text{ cm} \times 2.5\text{ cm}$) of an untreated Kleenex tissue. The information obtained from this control gives the number of infectious virus units that can be recovered from an untreated one inch square wipe following the virucidal testing procedure. The inoculated tissue culture tubes are examined for seven days for evidence of viral infection.

The endpoint of a virucidal test for a given wipe is that dilution of virus which infects actually or is calculated to infect only one of the two inoculated tubes. This number is defined as tissue culture infective dose, or TCID_{50} . The results of the virucidal activity of a given wipe are usually given as the "log difference" between the common log of the TCID_{50} result of the treated sample subtracted from the common log of the TCID_{50} of the untreated sample.

The virucidal efficacy of the sample may be derived from the "log difference" in the following manner:

$$\text{Virucidal efficacy} = \left(\frac{X - Y}{X} \right) 100\%$$

Where:

X = the initial concentration of the virus (infectious units/0.1 ml) of untreated sample used as control.

Y = the final concentration of the virus (infectious units/0.1 ml) of the treated sample.

The following examples explain the computation procedure. In the experiments, the final virus concentration was always less than or equal to $10^{2.3}$ infectious units/0.1 ml. For the majority of the results, the final virus concentration was less than $10^{2.3}$. With an initial virus concentration of $10^{6.3}$, this would signify a log difference greater than 4 and a "kill" of greater than 99.99%.

1. Initial concentration: $X=10^{6.3}$
 Final concentration: $Y=10^{2.3}$
 Log difference= $(\log 10^{6.3}-\log 10^{2.3})=4$

$$\begin{aligned}\text{Virucidal Efficacy} &= \left(\frac{10^{6.3} - 10^{2.3}}{10^{6.3}} \right) \times 100\% \\ &= \left(\frac{10^{2.3}(10^4 - 1)}{10^{6.3}} \right) \times 100\% \\ &= 99.99\%\end{aligned}$$

2. Initial concentration: $X=10^{4.8}$
 Final concentration: $Y=10^{2.3}$
 Log difference= 2.5

$$\begin{aligned}\text{Virucidal Efficacy} &= \left(\frac{10^{4.8} - 10^{2.3}}{10^{4.8}} \right) \times 100\% \\ &= 99.7\%\end{aligned}$$

The procedure outlined above is in conformity with standard microbiological assay techniques. It yields reliable and reproducible results within the limits of variability associated with biological experiments.

15 Assay results

The results are shown in Tables I, II, and III. The data in Table I show that simple organic carboxylic acids such as citric, malic, tartaric, succinic and substituted derivatives thereof (e.g. 2-bromo-succinic), and benzoic acid and its substituted derivatives (salicylic acid), used in a facial tissue in suitable concentrations, are highly virucidal against rhinovirus 16 and parainfluenza 3.

- 20 Furthermore, the data in Table I show that, when used in conjunction with a surfactant such as Aerosol OT or sodium dodecyl sulfate, the concentrations of the acids in the facial tissue may be lowered without sacrificing virucidal efficacy.

- 25 Table II lists the results of experiments with acid mixtures chosen from the group citric, benzoic, succinic, and malic. The data show that the facial tissues treated with the acid mixtures are virucidal against rhinovirus 16 and parainfluenza 3. The data on Table II show that the facial tissue impregnated with a mixed acid system such as citric and malic and an appropriate surfactant such as SDS, is efficacious against rhinovirus 16, 1A and 86 and adenovirus 5. As these examples demonstrate, in accordance with the present invention, simple organic acids such as citric/malic/succinic, when used in conjunction with a suitable surface-active agent such as SDS, are highly virucidal against common respiratory viruses of which rhinovirus 16, 1A and 86, parainfluenza 3, and adenovirus 5 are typical examples. In addition, products using facial tissues as the means of deployment of the virucidal compositions mentioned are highly effective.

- 35 The significance of the invention resides in the fact that it provides the basis for interrupting the chain of infection caused by respiratory viruses. As viruses do not replicate outside the host cell, the degree of inactivation demonstrated in the experiments offers a simple and practical means of reducing the virus concentration in the vicinity of a person infected with a respiratory virus. This, in turn, significantly reduces the potential of the infection to spread.

Table I
Virucidal efficacy of single acids against rhinovirus 16 and parainfluenza 3 virus (exposure time of one minute)

Example No.	Virucidal composition ^a	Surfactant ^a	Virucidal efficacy	
			Rhinovirus 16	Parainfluenza 3
1	Citric acid (23.2%)	None	>99.99%	>99.7%
2	Citric acid (18.7%)	None	>99.99%	
3	Citric acid (9.7%)	AOT ^b (1%), SDS ^c (1%)	99.99%	
4	Citric acid (9.4%)	SDS (1%)	>99.99%	>99.99%
5	Succinic acid (20%)	None	>99.99%	
6	Succinic acid (9.1%)	SDS (2%)	>99.99%	>99.99%
7	2-Bromosuccinic acid (10.4%)	SDS (1%)	>99.99%	
8	Malic acid (9.4%)	AOT (0.5%)	99.99%	>99.99%
9	Tartaric acid (15%)	None	>99.99%	
10	Benzoic acid (30%)	None	>99.99%	
11	Salicylic acid (18%)	None	>99.99%	
12	Salicylic acid (9%)	None	>99.99%	

^a The figures in parentheses represent percent chemical used based on the weight of the facial tissue.

^b Aerosol OT, the sodium salt of the 1,4-bis(2-ethylhexyl) ester of sulfosuccinic acid.

^c Sodium dodecyl sulfate.

Table II
Virucidal efficacy of mixed acids against rhinovirus 16 and parainfluenza 3 virus (exposure time of one minute)

Example No.	Citric acid	Virucidal composition*			Succinic acid	Surfactant ^a	Virucidal efficacy	
		Benzoic acid	Malic acid	acid			Rhinovirus 16	Parainfluenza 3
13	10.7	0.2	—	—	—	AOT ^b (1)	>99.99	>99.97
14	10.3	0.2	—	—	—	AOT (1)	>99.99	>99.97
15	10.1	0.2	—	—	—	AOT (1)	>99.99	>99.97
16	7.1	0.2	—	—	—	AOT (1)	>99.99	>99.99
17	8.8	0.2	—	—	—	AOT (1)	>99.99	>99.99
18	10.3	—	—	5.2	5.2	AOT (1)	>99.99	>99.97
19	10.0	—	—	5.0	5.0	AOT (1)	>99.99	>99.97
20	10.0	—	—	5.0	5.0	AOT (1)	>99.99	>99.97
21	10.4	—	5.2	—	—	AOT (1)	>99.99	>99.99
22	10.5	—	5.3	—	—	AOT (1)	>99.99	>99.97
23	10.3	—	5.2	—	—	AOT (1)	>99.99	>99.97
24	10.2	—	5.1	—	—	AOT (1)	>99.99	>99.97
25	11.1	—	5.6	—	—	AOT (0.5)	>99.99	>99.7
26	10.6	—	5.3	—	—	AOT (1)	>99.99	>99.7
27	11.1	—	5.6	—	—	AOT (0.5)	>99.99	>99.7
28	10.6	—	5.3	—	—	AOT (1)	>99.99	>99.70
29	4.8	—	4.8	—	—	AOT (1)	>99.99	>99.99
30	13.8	—	—	5.0	5.0	TX 100 ^c (2)	>99.99	>99.99
31	5.7	—	5.7	—	—	SDS ^d (2)	>99.97	>99.90
32	—	0.2	9.7	—	—	SDS (2)	99.97	>99.90

^a The figures in parentheses represent percent chemical used based on weight on the facial tissue.

^b Aerosol OT[®]

^c Triton X-100[®]

^d Sodium dodecyl sulfate

* The figures represent per cent chemical used based on the weight of the facial tissue.

Table III
Virucidal efficacy of mixed acids and SDS against rhinovirus 16, rhinovirus 1A, rhinovirus 86 and adenovirus 5 (exposure time of one minute)

Example No.	Virucidal composition ^a			Virucidal efficacy		
	Citric acid	Malic acid	Surfactant SDS ^b	Rhinovirus 16	Rhinovirus 1A	Rhinovirus 86
33	10.8	5.5	2.2	>99.99	—	—
34	11.2	5.7	2.3	>99.99	—	—
35	11.4	5.8	2.3	>99.99	—	—
36	10.8	5.5	2.2	>99.99	—	—
37	11.2	5.7	2.3	>99.99	—	—
38	10.0	5.0	2.0	>99.99	>99.9	>99.9
						Adenovirus 5
						99.90
						99.90
						99.70
						99.99
						99.99
						99.90

^a The figures represent percent chemical used based on the weight of the facial tissue.

^b Sodium dodecyl sulfate.

In order to more specifically illustrate the improved effects obtained in accordance with the invention, additional examples were carried out varying the concentration of selected acid compositions and measuring virucidal activity at one and five minutes. These results are summarized in Table IV. In general, the acid compositions within the scope of the invention are virucidally effective to a high degree e.g., in the case of rhinoviruses or parainfluenzas, they produce a log drop of 2 or greater inactivation in one minute or less. For adenoviruses the time will be five minutes or less. In general, the degree of inactivation is greater after five minutes than after one minute as would be expected. Certain minor inconsistencies appear in the reported results due to the margin of error and the nature of the test procedure. It will be recognized by those skilled in this art that effectiveness is also influenced by the amount of the composition available for contact with the virus which, in turn, depends on the nature of the carrier. For example, as shown in Table IV, below, a relatively thick carrier with large voids such as wool may be ineffective unless treated with large amounts of the composition. On the other hand, a lightweight, relatively closed structure such as tissue or nonwoven material will require less of the composition. Based on the tests described, however, the effectiveness of a given combination of composition and carrier may be determined. For example, as shown in Table IV, citric acid is effective at concentrations tested from 5% to 10% add-on. The procedure used is described below.

For these examples TCID₅₀ results were obtained using WI-38 cells of low passage from Flow Laboratories, Inc. which were initially passed at least once to insure growth potential. The bottles were then split 1:2 and seeded in 96-well cluster tissue culture plates with a flat bottom growth area of 0.32 cm² obtained from M A Bioproducts. The cells were incubated at 37°C in 5% CO₂ and, after 24 hours, were usually 80 to 90% sheeted and normal in appearance before use in the assay. The medium (2% MM) used for both dilutions and maintenance of the cells was MEM Eagles with Earles BSS (with glutamine, gentamycin and 2% fetal calf serum added). Rhinovirus 1A was obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. A vial was grown in WI-38 cells and harvested after showing 4⁺ cytopathogenic effect (CPE) at 2 days post inoculation. The virus was harvested, aliquoted, and frozen at -70°C and later titered in WI-38 cells in 96-well cluster plates.

For the assay, the medium was removed from the plates by placing sterile gauze between the plate and the cover and turning the plate over. All six wells used received 0.1 ml of 2% MM. To the wells which were to be used as cell controls, another 0.1 ml of 2% MM was added. To the cells which were to receive the compounds, 0.1 ml of the appropriate dilution of material was added to each of six wells. The stock virus was mixed 1:1 with 2% MM for the initial dilution. One hundred microml. of this virus dilution were then added to a treated disc in a petri dish. The virus was applied evenly over a tissue disc using a microliter syringe. The virus was allowed to remain on the disc for 1 minute or 5 minutes, then 5 ml of 2% MM was added to the disc in the Petri dish and the disc was slightly agitated. The disc and the solution were removed and placed in a sterile tube and agitated by vortexing for 30 seconds, representing the first dilution. Three ten-fold dilutions were made from the original tube and 0.1 ml of all four dilutions were added to the mono-layered WI-38 cells. Six wells were used for each dilution. Untreated controls were tested at 1 and 5 minutes, with and without virus and a virus titration was also run with each assay. The plates were reincubated at 37°C in 5% CO₂ for the duration of the test.

Acids such as sulfamic and phosphoric were also found to be virucidal. However, these acids have been found to degrade carriers such as tissue.

Table IV

Example	Acid	Concen- tration % add-on	Surfactant SDS—%	One Minute		Five Minutes		Virucidal efficacy (% Kill)	
				TCID ₅₀ (Log ₁₀) treated tissue	≈Log drop vs. control	Log ₁₀ (TCID ₅₀) treated tissue	≈Log drop vs. control	One min.	Five min.
39	Glycolic	12	—	<2.0	>3.25	<2	>2.75	>99.94	>99.82
40	"	9	—	<2.0	>3.25	<2	>2.75	>99.94	>99.82
41	"	2.4	—	<2.0	>3.25	<2	>2.75	>99.94	>99.82
42	Salicylic	7.2	—	<2.0	>2.33	<2	>2.75	>99.5	>99.8
43	"	5.4	—	<2.0	>2.33	<2	>2.75	>99.5	>99.8
44	"	3.6	—	≥5.17	0	4.67	0.5	0	68
45	"	1.4	—	≥5.25	0	≥5.4	0	0	0
46	Succinic	9.2	—	<2.0	>3.25	<2	>2.75	>99.94	>99.82
47	"	6.9	—	<2.0	>3.25	<2	>2.75	>99.94	>99.82
48	"	4.6	—	<2.0	>3.0	<2	>3.17	>99.9	>99.93
49	"	1.8	—	3.9	0.8	3.9	0.4	84	60
50	Malic	10.5	—	<2.0	>3.25	<2.0	>2.75	>99.94	>99.82
51	"	7.9	—	NA	NA	<2.0	>2.75	NA	>99.82
52	"	5.2	—	<2.0	>3.25	<2.0	>2.75	>99.94	>99.82
53	"	2.1	—	2.38	2.87	<2.0	>2.75	99.9	>99.82
54	2-Bromo-Succinic	2.0	—	3.33	1.92	<2.0	>2.75	98.8	>99.8
55	"	10.2	2.0	2.5	2.5	2.5	2.67	99.7	99.8
56	Tartaric	11.7	—	<2.0	>3.25	<2.0	>2.75	>99.94	>99.82
57	"	8.8	—	<2.0	>3.25	<2.0	>2.75	>99.94	>99.82
58	"	5.9	—	<2.0	>3.25	<2.0	>2.75	>99.94	>99.82
59	"	2.3	—	<2.0	>3.25	<2.0	>2.75	>99.94	>99.82
60	Maleic	6.8	—	<2.0	>3.25	a	a	>99.94	a
61	"	4.5	—	<2.0	>3.25	≤2.0	≥2.75	>99.94	≥99.8
62	"	1.8	—	2.25	≥3.00	<2.0	>2.75	>99.9	>99.8
63	Aconitic	9.0	—	<2.0	>3.25	<2.0	>2.40	>99.94	>99.6
64	"	6.8	—	≤2.0	≥3.25	<2.0	>2.75	≥99.94	>99.8
65	"	1.8	—	3.40	1.85	3.50	1.25	98.6	94
66	Citric	10.0	—	<2.0	>3.25	<2.0	>2.75	>99.94	>99.8 (2)
67	"	7.5	—	≤2.0	>3.25	<2.0	>2.40	≥99.94	>99.6
68	"	5.0	—	≤2.0	≥3.25	≤2.0	>2.75	≥99.94	>99.8 (2)
69	"	2.0	—	3.75	1.0	<2.0	>2.4	90	>99.6
70	Phosphoric	5.0	—	<2.0	>3.0	<2.0	>3.17	>99.9	>99.93
71	"	3.8	—	≤2.0	≥3.0	<2.0	>3.17	≥99.9	>99.93
72	"	2.5	—	≤2.0	≥3.0	<2.0	>3.17	≥99.9	>99.93
73	"	1.0	—	4.25	0.75	4.40	0.77	82	>83
74	Citric/Malic	10.0/5.0	—	<2.0	>1.75	<3.0	>1.40	>98.2	>96

Table IV (continued)

Example	Acid	Concen- tration % add-on	Surfactant SDS—%	TCID ₅₀ (Log ₁₀) treated tissue	One Minute		Five Minutes		Virucidal efficacy (% Kill)	
					TCID ₅₀ (Log ₁₀) treated tissue	≈Log drop vs. control	Log ₁₀ (TCID ₅₀) treated tissue	≈Log drop vs. control	One min.	Five min.
75	"	"	—	<2.0	>3.0	>3.17	<2.0	>3.17	>99.9	>99.93
76	Wool substrate BW=174.4 mg/ sq. in)	"	0.6 mg/in ²	4.6	0.4	NA	NA	NA	60.0	NA
77	Meltblown poly- propylene face- mask BW=52.5 mg/in ²	"	0.6 mg/in ²	a	a	1.6	<3.0	1.6	NA	>97.5

Note: a in some cases particularly with addition of surfactant, cytopathic effects prevented useful data from being obtained. Such effects are described in Lennette, et al. *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*, 1979, 5th Ed., p. 67.

Table V

Example	Acid	μ Mole/in ² *	Acid add-on %	Surfactant add-on %	Virocidal activity (% Inactivation of rhinovirus 16)	
					1 Min.	5 Min.
78	Sulfamic	15.6 (100.6)	5	—	99	>99.997
79	"	46.8 (301.9)	15	—	99.997	>99.997
80	"	15.6 (100.6)	5	—	99	99
81	"	15.6 (100.6)	5	SDS 2%	99	>99.99

* Figures in brackets in (μ mol/cm²)

Because such acids are soluble in water, they can be applied to many substrates from an aqueous solution with great ease either by dipping, coating, or other conventional means such as spraying or gravure printing. When applied to the substrates, the composition is applied in an amount sufficient to provide virucidal activity as defined herein. While the lower effective limit for the acids has not been precisely determined, in general, for a substance such as facial tissue having a basis weight in the range of 23 to 31 lbs./2880 ft.² (0.086 to 0.116 kg/m²) (3 ply), there should be a pick-up of at least about 2 percent and preferably about 5 percent of acids such as citric on a dry basis. Other substrates such as nonwovens may be utilized as well.

With respect to other substrates, those with high wettability are preferred. As shown, low virucidal effect with substrates such as wool is believed due to inability to penetrate the substrate with the virucidal composition.

When mixtures of acids are employed, they may be in any proportion, but preferably the mixtures contain at least about 0.2 to 10% of each acid based on the weight of the substrate after drying.

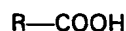
When surfactants are included, they are preferably selected from the group of anionic surfactants and included in the amount of about 0.05 to 5% based on the weight of the substrate after drying.

In the application of the virucidally active organic acids defined herein in other substrates or carriers such as lotions, mouthwash, creams, sprays, polishes and the like, the virucidally effective amount may be determined readily by application of the procedures set forth herein. For example, a log drop of 2 or more would mean that 99 percent or more of the host viruses are inactivated upon contact with the antiviral acid compositions described and claimed herein.

Thus, it is apparent that the virucidal product in accordance with the invention may, under conditions of normal use, fully satisfy the objectives and advantages as set forth in the previous paragraphs.

Claims

1. A method for interrupting or preventing the spread of respiratory viruses which comprises contacting a virus-containing area with a virucidally effective amount of a virucidally active composition comprising one or more virucidal acids which are essentially non-toxic and non-irritating to human or animal tissue selected from acids of formula



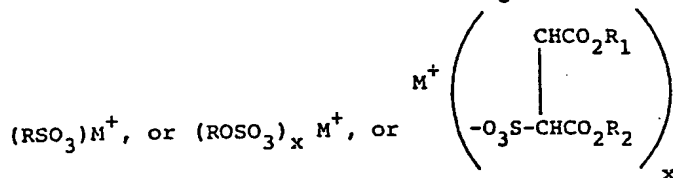
(wherein R represents a lower alkyl, substituted lower alkyl, carboxy lower alkyl, carboxy hydroxy lower alkyl, carboxy halo lower alkyl, carboxy dihydroxy lower alkyl, dicarboxy hydroxy lower alkyl, lower alkenyl, carboxy lower alkenyl, dicarboxy lower alkenyl, phenyl or substituted phenyl group).

2. A method as claimed in claim 1 wherein the said virus containing area is an area of human or animal tissue.

3. A method as claimed in either of claims 1 and 2 wherein said acid is selected from citric, malic, succinic and benzoic acids and substituted derivatives thereof.

4. A method as claimed in any one of claims 1 to 3 wherein said composition further comprises a surfactant selected from nonionic, cationic and anionic surfactants.

5. A method as claimed in claim 4 wherein said surfactant is selected from polyoxyethylenated alkyl phenols, polyoxyethylenated sorbitol esters, quaternary ammonium salts and sulfuric acid ester salts, alkyl sulfonic acid salts and sulfosuccinic ester salts having the structure:



(wherein M⁺ is a mono, di or trivalent metal cation or an ammonium or substituted ammonium ion; x is an integer; R is an alkyl group; and R₁ and R₂, which may be the same or different, are straight or branched chain aliphatic groups).

6. A method as claimed in claim 4 wherein said surfactant is selected from the sodium salt of 1,4-bis(2-ethylhexyl) ester of sulfosuccinic acid and sodium dodecyl sulfate.

7. A method as claimed in any one of the preceding claims wherein said one or more virucidal acids is a mixture of

- (a) citric and malic acids;
- (b) citric and benzoic acids;
- (c) citric and succinic acids;
- (d) malic and benzoic acids;
- (e) malic and succinic acids; or
- (f) succinic and benzoic acids.

8. A virucidal composition comprising a physiologically acceptable carrier containing a virucidally

effective amount of one or more acids which are non-toxic and non-irritating to human or animal tissue in the amount used selected from acids of formula

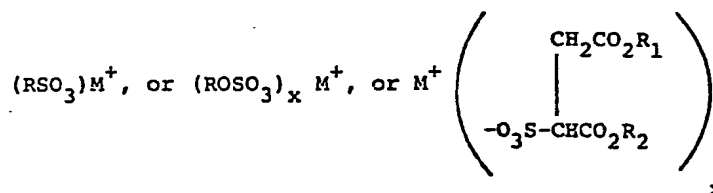


(wherein R represents a lower alkyl, substituted lower alkyl, carboxy lower alkyl, carboxy hydroxy lower alkyl, carboxy halo lower alkyl, carboxy dihydroxy lower alkyl, dicarboxy hydroxy lower alkyl, lower alkenyl, carboxy lower alkenyl, dicarboxy lower alkenyl, phenyl or substituted phenyl group). 5

9. A composition as claimed in claim 8 wherein said acid is selected from citric, malic, succinic and benzoic acids and substituted derivatives thereof.

10. A composition as claimed in either of claims 8 and 9 further comprising a surfactant selected from nonionic, cationic and anionic surfactants. 10

11. A composition as claimed in claim 10 wherein said surfactant is selected from polyoxyethylenated alkyl phenols, polyoxyethylenated sorbitol esters, quaternary ammonium salts and sulfuric acid ester salts, alkyl sulfonic acid salts and sulfosuccinic ester salts having the structure:



15 (wherein M^+ is a mono, di or trivalent metal cation or an ammonium or substituted ammonium ion; x is an integer; R is an alkyl group; and R_1 and R_2 , which may be the same or different, are straight or branched chain aliphatic groups). 15

12. A composition as claimed in claim 11 wherein said surfactant is selected from the sodium salt of 1,4-bis(2-ethylhexyl) ester of sulfosuccinic acid and sodium dodecyl sulfate.

20 13. A composition as claimed in any one of claims 8 to 12 wherein said one or more acids is a mixture of 20

(a) citric and malic acids;

(b) citric and benzoic acids;

(c) citric and succinic acids;

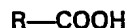
25 (d) malic and benzoic acids; 25

(e) malic and succinic acids; or

(f) succinic and benzoic acids.

14. A virucidal composition substantially as herein described comprising carboxylic acids contained in a physiologically acceptable carrier.

30 15. A virucidal product comprising a web substrate containing a virucidally effective amount of a composition comprising one or more acids which are non-toxic and non-irritating to human or animal tissue in the amount used and are selected from acids of formula 30



(wherein R represents a lower alkyl, substituted lower alkyl, carboxy lower alkyl, carboxy hydroxy lower alkyl, carboxy halo lower alkyl, carboxy dihydroxy lower alkyl, dicarboxy hydroxy lower alkyl, lower alkenyl, carboxy lower alkenyl, dicarboxy lower alkenyl, phenyl or substituted phenyl group). 35

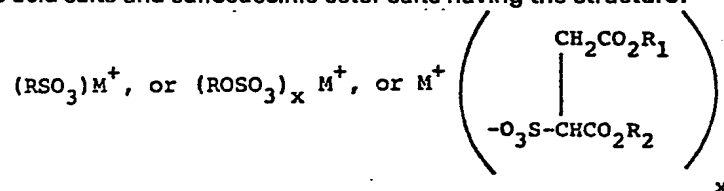
16. A product as claimed in claim 15 wherein said substrate is selected from cellulose tissue, nonwoven fabrics and textile materials.

40 17. A product as claimed in either of claims 15 and 16 wherein said acid is selected from citric, succinic and benzoic acids and substituted derivatives and mixtures thereof, said acid being present in an amount of about 2% or more by weight relative to the weight of the substrate. 40

18. A product as claimed in any one of claims 15 to 17 wherein said substrate is a cellulosic facial tissue.

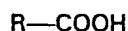
45 19. A product as claimed in claim 15 wherein said composition further comprises a surfactant selected from nonionic, cationic and anionic surfactants. 45

20. A product as claimed in claim 19 wherein said surfactant is selected from polyoxyethylenated alkyl phenols, polyoxyethylenated sorbitol esters, quaternary ammonium salts and sulfuric acid ester salts, alkyl sulfonic acid salts and sulfosuccinic ester salts having the structure:



(wherein M^+ is a mono, di or trivalent metal cation or an ammonium or substituted ammonium ion; x is an integer; R is an alkyl group; and R_1 and R_2 , which may be the same or different, are straight or branched chain aliphatic groups).

21. A product as claimed in claim 19 wherein said surfactant is selected from the sodium salt of
 5 1,4-bis(2-ethylhexyl) ester of sulfosuccinic acid and sodium dodecyl sulfate. 5
22. A product as claimed in any one of claims 15 to 21 wherein said one or more acids is a mixture of
- 10 (a) citric and malic acids;
 (b) citric and benzoic acids;
 (c) citric and succinic acids; 10
 (d) malic and benzoic acids;
 (e) malic and succinic acids; or
 (f) succinic and benzoic acids.
23. A virucidal product substantially as herein described comprising a web substrate containing a
 15 virucidally effective amount of one or more carboxylic acids. 15
24. Acids of formula



- (wherein R represents a lower alkyl, substituted lower alkyl, carboxy lower alkyl, carboxy hydroxy lower alkyl, carboxy halo lower alkyl, carboxy dihydroxy lower alkyl, dicarboxy hydroxy lower alkyl, lower
 20 alkenyl, carboxy lower alkenyl, dicarboxy lower alkenyl, phenyl or substituted phenyl group), if desired 20
 in composition with nonionic, cationic or anionic surfactants, for use in a method of treatment of human or animal bodies to reduce or eliminate spread of respiratory viruses therebetween.